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SURVIVABILITY AND INFECTIVITY OF VISCEROTROPIC *LEISHMANIA TROPICA* FROM OPERATION DESERT STORM PARTICIPANTS IN HUMAN BLOOD PRODUCTS MAINTAINED UNDER BLOOD BANK CONDITIONS

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Abstract. To assess the potential for leishmaniasis being transmitted through blood transfusion, we studied the survival of *Leishmania* in blood products under blood bank storage conditions. We report that *L. tropica*– or *L. donovani*–contaminated transfusable blood products are a risk to the blood supply for at least 25 days postdonation under blood bank general conditions. The blood components that have been implicated are whole blood, packed red blood cells, platelet concentrate, and frozen-deglycerolized red blood cells, but not, as would be expected, fresh frozen plasma. Blood units containing four infected monocytes per milliliter of blood with a mean of three amastigotes per monocyte contain viable parasites for 15 days under blood bank storage conditions. Furthermore, animal studies showed the presence of parasites in the blood of cutaneously infected animals and the possibility of transmitting the disease to healthy experimental animals by blood transfusion from infected animal donors. Three of three BALB/C mice showed metastasis to the lower extremities and face after they received 0.25 ml of blood from a CPDA-1 bag seeded with 1.5×10^5 amastigotes per ml of blood kept under blood bank conditions for 30 days. This proves that *Leishmania* not only survives blood banking procedures and storage conditions but that the parasite retains its infectivity. The results of this study and the recent demonstration of *L. tropica*–infected monocytes in the blood of a patient returning from Southwest Asia suggests that transfusion-associated leishmaniasis can occur.

On November 12, 1991, the Department of Defense issued an advisory recommending that all individuals who had traveled to the Persian Gulf after August 1, 1990 be deferred as donors of transfusable blood products. This action was taken because *Leishmania tropica* was isolated from the bone marrow of seven of eight members of the Armed Forces returning from Operation Desert Storm with viscerotropic leishmaniasis.[1]

The risk of infection from transfusable blood products is proportional to the magnitude and duration of peripheral parasitemia in donors infected with *Leishmania*, the survivability of the parasite under blood bank storage conditions, and the infectivity of the blood so stored. Since viscerotropic disease was a newly recognized clinical presentation of *L. tropica* in 1991, none of these three factors were known. Moreover, the survivability and infectivity of *Leishmania* in blood under blood bank storage conditions was not known for any species.

There have been six cases of transfusion-acquired leishmaniasis reported.[2–5] Two donors were asymptomatic. Furthermore, although lower rates have been reported, in some endemic areas peripheral blood smears and/or hemocultures from patients with active kala-azar are positive for parasites in 75–95% of cases.[6–8] Although the eight patients indicated above have a lower parasite burden than do classic kala-azar patients, parasites were consistently isolated from bone marrow. In addition, blood product recipients include immunosuppressed and critically ill patients who are at risk for serious illness from even a small inoculum.

In the present studies, we report the survivability and infectivity of viscerotropic *L. tropica* from Desert Storm participants in human blood maintained under blood bank conditions. The ease with which *L. tropica* and *L. donovani* survived and were infective suggests that there is a reasonable possibility that blood products from infected persons, if parasitemic, may transmit leishmaniasis.

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MATERIALS AND METHODS

Parasites

The following strains were used in these experiments: *L. major* (MHOM/SA/91/WR2036), *L. tropica* (MHOM/SA/91/WR1063), and *L. donovani* (MHOM/IN/80/DD8). Cultures were initiated with a stabilate of the primary isolate in the case of WR2036 and WR1063, and with a cryopreserved aliquot of the original stock in the case of DD8. Strain WR2036 was isolated from a 54-year-old male National Guard soldier who had been deployed to Saudi Arabia and returned with multiple cutaneous lesions (four on the left leg, three on the left side of the abdomen, three on the right side of the back, and one on the left side of the back). Isolate WR1063 was recovered from the bone marrow of a 30-year-old active duty male who presented with the abrupt onset of fever, malaise, fatigue, and abdominal pain. During Operation Desert Storm, he had served in an airborne unit located near a town on the Persian Gulf coast. Periodically, the soldier's unit moved out into the desert on bivouacs for two or three days at a time. His physical findings at the time of the confirmatory culture were weight loss and adenopathy. Laboratory analysis revealed a hematocrit of 37%, a white cell count of 10,500/mm^3, a platelet count of 160,000/mm^3, and a total protein value of 8.0 g/dl. Liver enzymes were elevated, with an aspartate aminotransferase level of 98 IU/L (normal range 10–50 IU/L) and an alanine aminotransferase level of 210 IU/L (normal range 5–42 IU/L). The patient was treated with 20 mg/kg/day of Pentostam for 30 days as a daily intravenous infusion, with resolution of signs and symptoms of the illness.

In vitro culture

Promastigotes of the above-listed strains were cultured at 25°C in complete Schneider's medium (Schneider's *Drosophila* medium [Gibco, Grand Island, NY] supplemented with 20% heat-inactivated fetal calf serum and 100 μg/ml of gentamicin). Cells were maintained in logarithmic phase by seeding at 2×10^6/ml when cultures approached 2×10^7/ml. Stationary phase promastigotes were obtained when cultures approached 7×10^7/ml (WR2036 and WR1063) and 5×10^7/ml (DD8). Growth rate determinations were made by counting cell numbers daily in a hemocytometer.

Survival of L. donovani *and viscerotropic* L. tropica *extracellular amastigotes and promastigotes in whole blood*

A predetermined number of *L. tropica* WR1063 amastigotes obtained from the footpads of infected BALB/C mice, as well as *L. tropica* or *L. donovani* DD8 stationary phase promastigotes, were introduced into freshly donated human blood from healthy volunteers. Subsequently, the blood was maintained at the recommended temperature/time for storing blood products (4°C for 35 days for whole blood, 24°C for five days for platelet cells). At intervals specified below, a sample of blood from each temperature was removed, and the number of viable parasites per milliliter was determined by two methods: by counting viable cell numbers in a hemocytometer, and by culturing 200 μl of the seeded blood in 1.0 ml of complete Schneider's medium (25°C) and assessing viability microscopically.[9] Whole blood kept at 4°C was tested daily for five days and then every five days for 35 days; blood kept at 24°C was tested at the end of days 1, 2, 3, 4, and 5.

Survival and quantification of L. donovani *and viscerotropic* L. tropica *intramonocyte amastigotes in whole blood and human blood products*

Mononuclear cells were purified by density sedimentation on Lymphocyte Separation Medium-LSM (Organon Teknika, Durham, NC) from 50 ml of blood. Monocytes were purified by adherence to six-well plastic culture dishes (1 hr at 37°C in 5% CO_2) and cultured as adherent cells in 10% heat-inactivated human serum in RPMI 1640 tissue culture medium. Cultures were infected with WR1063 or DD8 stationary phase promastigotes at a ratio of 4:1, which was previously determined to induce > 90% infection of monocytes. After culture with parasites for 16 hr, monocytes were scraped from the wells and cultured as a nonadherent cell pellet in supplemented RPMI 1640 medium in polypropylene tubes. The percent/total number of infected monocytes and the number of amastigotes per infected monocyte were determined by light microscopic examination of Dif-Quik (Baxter, Mi-

ami, FL)–stained cytospin preparations of monocytes released from the plastic by scraping. The number of viable parasites in these monocytes was independently determined by staining of monocyte suspensions with fluorescein diacetate/ethidium bromide,[10] and by analysis of amastigote-to-promastigote conversion from cultures of infected monocytes serially diluted in Schneider's medium.

To determine the survivability of intramonocyte amastigotes in transfusable blood products stored under blood bank conditions, monocytes were purified from 50 ml of blood from paid, parasite-free donors, infected, and the intensity of infection determined as described above. One day later, an additional 400 ml of blood was collected from the same donors in an 800-ml CPDA-1 quadruple blood bag (Baxter, Deerlake, IL). A known number of each donor's infected monocytes were added in a total volume of 1 ml of autologous blood to the 400-ml pack of blood. Within the time period required by blood bank procedures, the donated units were fractionated into the different blood components and stored as indicated (packed red blood cells [RBC] at 4°C, platelet cells at 24°C, fresh frozen plasma at −70°C, RBC frozen by glycerolization [frozen RBC] at −70°C), or permitted to remain as whole blood at 4°C. At determined intervals, a sample from the different components was removed and tested in triplicate for the presence of viable organisms by the methods previously described. Platelet cells were tested at the end of days 1, 2, 3, 4, and 5. Packed red blood cells and whole blood were tested daily for five days and then every five days for five weeks. An aliquot of frozen RBC and frozen plasma were thawed after five and 35 days and tested.

A pilot study was conducted to determine the detection limit for intramonocyte amastigotes in complete Schneider's medium by culturing serial dilutions of a known number of amastigotes within monocytes. This pilot study was necessary because survivability was measured by culture (transformation of viable intramonocyte amastigotes into promastigotes) in complete Schneider's medium.

In vivo blood transfusion experiments and isolation of amastigotes from the blood of laboratory animals

Six–eight-week-old female BALB/C mice, and 65-g male golden hamsters were obtained commercially and maintained as outlined by the *Guide for the Care and Use of Laboratory Animals* and the Animal Welfare Act, AR 70-18.[11] Mouse and hamster chow and water was supplied ad libitum. The hair was clipped from the infection site on the rump prior to infection with 5×10^6 stationary phase promastigotes (*L. tropica* WR1063 for hamsters and *L. major* WR2036 for BALB/C mice). After two months, BALB/C mice and hamsters with ulcerated lesions > 50 mm^2 were selected for the studies. The animals were bled by the intracardiac route, and the blood (approximately 0.5 ml/animal) was cultured in 10 ml of complete Schneider's *Drosophila* medium for 30 days. Blood cultures were observed daily by light microscopy for the presence of promastigotes to determine the presence of *Leishmania*.

Two groups of five BALB/C mice were transfused via the caudal vein using a 1-cc syringe with 0.5 ml of blood from animals infected four months previously with *L. major* and presenting with cutaneous lesions. One group received blood stored for 30 days at 4°C. The other group of naive recipients received blood immediately after it was withdrawn from the infected animals. An additional group of three healthy BALB/C mice were transfused with 0.25 ml of whole human blood spiked with *L. tropica*–infected human monocytes and kept at 4°C for 30 days (blood pack CPDA-1) to determine parasite infectivity. The animals were examined at weekly intervals for four months after the blood transfusion for signs of infection such as cutaneous lesions, hepatosplenomegaly, or metastasis. At the end of the four months, the transfused animals were killed and liver and spleen samples were cultured in Schneider's medium for 30 days. Both Giemsa staining and the indirect immunofluorescent monoclonal antibody assay, using a genus-specific fluorescent-labeled monoclonal antibody (WR2A), were used to identify amastigotes present in liver and spleen samples from transfused animals to determine infection.[12] At least 100 microscopic fields (43×) were examined before rendering a report as negative. At the end of the experiment, all surviving animals were killed with CO_2.

Withdrawal of blood from human volunteers and animal husbandry were performed according to protocols approved by appropriate ethical review bodies. All subjects enrolled in the study voluntarily agreed to participate and gave written informed consent.

TABLE 1

Survival of viscerotropic Leishmania tropica *in human whole blood**

Time (days)	% parasite survival at 4°C: Promastigotes	Free amastigotes	Three amastigotes/monocyte	% parasite survival at 24°C: Promastigotes	Free amastigotes	Three amastigotes/monocyte
0				100	100	100
5	85	100	90	30	45	90
10	30	76	90			
15	5	42	90			
20	5	13	85			
25	0	5	45			
30		0	16			
35			0			

* Peripheral blood was collected, divided into 1-ml aliquots, and seeded with 1×10^6 promastigotes, free amastigotes, or intracellular amastigotes (333,333 infected human monocytes with three amastigotes/monocyte) and maintained at 4°C and 24°C. Studies at 4°C were performed for 35 days because whole blood and components kept at this temperature are maintained for 35 days. Studies at 24°C were limited to five days because blood components (platelets) kept at 24°C are only stored for five days. On the days indicated, parasite survival was determined by adding 200 µl of seeded blood (2×10^5 cells) to 1 ml of Schneider's medium and examining the Schneider's cultures each day for the next 45 days (25°C) for viable parasites. Percentage survival was determined by dividing the number of promastigotes on the first day in which organisms were detected by the total number of seeded organisms.

TABLE 2

Survival of viscerotropic Leishmania tropica *and* L. donovani *in human blood products under blood bank conditions**

Time (days)	RBC 4°C	PC 24°C	Frozen RBC −70°C	FFP −70°C	WB 4°C
0		+			
5	+	+	+	−	+
10	+				+
15	+				+
20	+				+
25	+				+
30	−				+
35	−		+	−	−

* Human monocytes were infected with *L. tropica* stationary-phase promastigotes at a ratio of four promastigotes per one monocyte. At 16-hr postinfection, there was a mean of three amastigotes per infected monocyte at a final concentration of 6.3×10^6 infected monocytes/ml. One unit of blood (400 ml of autologous blood) was seeded with 3.3 ml of the infected monocytes (approximately 6.2×10^7 amastigotes), which resulted in ~1.5×10^5 amastigotes/ml of blood. Within the appropriate time periods, the blood was divided into packed red blood cells (RBC), platelet cells (PC), red blood cells frozen by glycerolization (frozen RBC), fresh frozen plasma (FFP), or left as whole blood (WB). The components were stored according to blood bank standards for transfusable blood. Infected cells were cultured after storage (0–35 days) in Schneider's medium in triplicate at two different volumes (50 µl of blood in 950 µl of Schneider's medium and 500 µl of blood in 9.5 ml of Schneider's medium). Platelets were maintained at 24°C for only five days in accordance with recommendations for the shelf-life of this blood component.

RESULTS

The survival of viscerotropic *L. tropica* stationary phase promastigotes, free amastigotes, and infected monocytes in whole blood kept under blood bank conditions is shown in Table 1. Viscerotropic *L. tropica* survived as intracellular parasites in monocytes for 30 days at 4°C, and for at least five days at 24°C. Intracellular parasites survived longer than did stationary phase extracellular promastigotes or free amastigotes.

The survival of viscerotropic *L. tropica* in human blood product units, as well as in units of whole blood, is shown in Table 2. The parasites survived as intracellular forms in monocytes for 25 days in the red blood cell fraction kept at 4°C, at least five days in the platelet fraction kept at 24°C, at least 35 days in the red blood cell fraction frozen with glycerol, and, as in the previous experiment, for 30 days in unprocessed whole blood left at 4°C. All fresh frozen plasma cultures were negative, probably because of the absence of cellular components. Identical experiments with *L. donovani* resulted in comparable survival data. As the only exception to the *L. tropica* survival data, *L. donovani* proved to be hardier, showing positive cultures through day 30 in packed red blood cell units, a difference of five days.

To define the minimum number of *L. tropica* needed to contaminate 1 ml of blood, serial dilutions with known numbers of intramonocytic amastigotes per milliliter of blood were cultured in whole blood at 4°C, and aliquots removed every five days to determine parasite viability. We had previously determined that the detection limit of intramonocyte *L. tropica* was 12 parasites (four infected monocytes with three amastigotes per monocyte) per ml of whole blood (Table 3). Table 4 shows the maximum dilution of organisms (i.e., the minimum number of organisms) that were viable after every five days of incubation. It was determined that one detectable viscerotropic *L. tropica* parasite survived for 15 days when whole blood was kept under blood bank conditions, but an inoculum of 256 organisms was required for a 35-day culture to have viable parasite(s).

Animal studies were carried out to determine 1) the presence of infected monocytes in the blood of cutaneously infected animals and 2) the possibility of transmitting the disease by blood transfusion from both infected animal donors and a seeded CPDA-1 bag of human whole blood kept for 30 days at 4°C under blood bank conditions. Viscerotropic *L. tropica* and cutaneous *L. major* were cultured from the blood of six of 10 cutaneously infected hamsters, and from five

TABLE 3

*Detection limits for intramonocyte amastigotes in human blood culture**

Amastigotes/monocytes	Culture results
100,000/33,333	+
10,000/3,333	+
1,000/333	+
100/33	+
50/16.5	+
25/8.25	+
12.5/4.12	+
6.25/2.06	–
3.12/1.03	–
1.56/0.51	–
0.78/0.25	–

* Triplicate cultures containing the indicated total number of amastigotes/monocytes were added to 1 ml of autologous blood, incubated at 25°C, and observed daily for 45 days by light microscopy for the presence of promastigotes before rendering a negative result. The triplicate results from all dilutions in both positive (+) and negative (–) cultures were identical.

TABLE 4

Quantitation of survival of viscerotropic Leishmania tropica *in human blood**

Maximal dilution	Detectable parasites at maximal dilution (calculated)	Incubation time (days)	% survival
1:5,120	1	15	100
1:2,560	2	20	50
1:1,280	4	20	25
1:640	8	25	12.5
1:320	16	25	6.25
1:160	32	30	3.12
1:80	64	30	1.56
1:40	128	35	0.78
1:20	256	35	0.39

* Sixty-three samples of infected monocytes (nine samples for each of the seven incubation periods) were prepared by diluting 200 μl of *L. tropica*–infected monocytes (6.4×10^4 amastigotes 2.1×10^4 infected monocytes) 1:20–1:5,120 with whole autologous blood, and suspending the mixture in a total of 1.0 ml of autologous blood. After each of the seven periods of incubation (days 5, 10, 15, 20, 25, 30, and 35), the 1-ml blood samples were added to 9 ml of complete Schneider's medium, cultured at 25°C and examined daily for 45 days to determine the first day on which promastigotes were visible. The Table depicts the sample containing the maximal dilution of infected monocytes (i.e., the minimal number of infected monocytes) in which promastigotes were visible on the indicated day of incubation. The number of detectable parasites in the sample was calculated from the number of parasites added to each 1.0-ml sample, and from the fact that only one out of 12 parasites in human blood is detectable via culture (Table 3). The percent survival at each time period was calculated by dividing the one parasite visualized at that time period by the number of detectable parasites added.

of nine cutaneously infected BALB/C mice, respectively. Cutaneous lesions (one case) or metastasis to the lower extremities (two cases) developed in three of the five mice transfused with a fresh 0.5-ml blood sample from lesion-positive BALB/C donors. In addition, *L. major* amastigotes were visualized and cultured from the liver and spleen of four of the five transfused animals. Similarly, four of the five mice transfused with blood stored at 4°C for 30 days showed metastasis to the face (two cases), metastasis to the upper extremities (one case), and metastasis to the lower extremities (one case). Amastigotes were visualized and cultured from the livers of all five transfused animals. Three of three additional animals who received 0.25 ml of blood from a CPDA-1 bag seeded with 1.5×10^5 *L. tropica* amastigotes per ml of blood and stored for 30 days also developed metastasis to the lower extremities or face. Thus, *Leishmania* not only survives under blood bank conditions, but the parasites retain their infectivity to healthy experimental animals.

DISCUSSION

The analyses of intracellular parasite survival in blood handled under conditions identical to that in a blood bank provides information useful on determining whether or not blood from donors infected with *L. donovani* or viscerotropic *L. tropica* pose a threat to blood recipients. In blood banks, whole blood and red blood cells are kept with specified anticoagulants and in specific plastic containers at 1–6°C for 35 days, platelet packs are kept at room temperature (22–24°C) for five days, and frozen red blood cells are kept at −65°C or lower for 10 years. We found that when identical materials and procedures are used, viscerotropic *L. tropica* within human monocytes qualitatively survives for 30 days in whole blood at 4°C, and for at least five days at 24°C. Furthermore, when red blood cells, platelet packs, or frozen red blood cell packs were derived from whole blood spiked with infected monocytes, organisms were viable for 25 days at 4°C in the red blood cells, for at least five days at 24°C in the platelet packs, and for at least 35 days at −70°C in the frozen red blood cell packs. The latter results indicate that mononuclear cell contamination of red blood cell or platelet packs results in sufficient numbers of infected monocytes to contaminate these blood products with viable *Leishmania*. Determination of the minimum number of organisms necessary to contaminate whole blood revealed that blood into which merely 12 organisms (four infected monocytes with a mean of three amastigotes per monocyte) were inoculated per ml had been contaminated for 15 days under blood banking storage conditions at 4°C. In experiments in which survival

of *L. tropica* and *L. donovani* were compared, there was no significant difference between the survival of *L. tropica* and that of *L. donovani* parasites, a species that normally visceralizes, and for which there is evidence for transmission through blood transfusion. This work indicates that *L. tropica* and *L. donovani* survive blood bank procedures and storage conditions.

Although it might be assumed that viable *Leishmania* could transmit disease to a naive individual, we tested this hypothesis experimentally in mice. Infected blood from mice infected subcutaneously with *L. major* and human blood seeded with *L. tropica* stored for 30 days at 4°C was infective to virtually all recipient animals.

There are six reported cases of transfusion-acquired leishmaniasis, all presumably due to *L. donovani*. In Sweden, blood from a healthy asymptomatic donor who had been outside the country in recent years was given to two newborns who became ill six months after receiving the transfusion. One of the newborns died and an autopsy revealed kala-azar; the second newborn was treated and survived.[4] A multiply transfused neonate in Belgium developed hepatosplenomegaly at 11 months, and a bone marrow aspiration showed amastigotes, although the infected donor was never identified.[5] Blood from an infected mother in China was donated to two daughters (four and six years old). The mother was diagnosed with kala-azar one month later and both daughters developed kala-azar nine and 10 months after receiving the transfusion; no other members of the household were infected.[2] Similarly, a man donated blood to two newborns in September 1955 after traveling to Spain one month earlier. He developed papular skin lesions and lymphadenopathy in November 1955; biopsies of both tissues showed amastigotes. One infant developed anemia and failure to thrive in November 1955, which progressed to kala-azar in June 1956. Diagnosis was confirmed by finding amastigotes in the bone marrow. The other infant remained well.[3]

This literature can be interpreted in two ways. On the one hand, transfusion-acquired leishmaniasis might be a rare event, seen in infants with immature immune systems after multiple transfusions. On the other hand, it could be argued that although transfusion-acquired leishmaniasis is most easily recognized in transfused infants from a nonendemic area, transfusion-acquired leishmaniasis could occur in any recipient receiving blood from anyone harboring *Leishmania*. To support this latter point of view, we note that *L. braziliensis* was isolated from the peripheral blood leukocytes of two patients with mucosal disease and patients with Old World cutaneous leishmaniasis, conditions usually not associated with parasitemia.[13–15] In addition, the true extent of transfusion-associated disease is probably underestimated because disease seen in endemic areas is attributed to sand fly bites. Nevertheless, transfusion-acquired cutaneous leishmaniasis has not been reported, and our data demonstrating the survival of *Leishmania* in blood under blood bank conditions should not be interpreted as a threat of transmission of cutaneous leishmaniasis in nature without additional supporting data. Lower numbers of circulating infected monocytes in patients with cutaneous disease versus those with visceral disease may explain why there are no reports of transfusion-acquired cutaneous leishmaniasis.

In general, the risk of transfusion-associated infection is determined by five factors: the prevalence of infection, the incidence of peripheral parasitemia in donors, the survivability of parasites stored in blood, the infectivity of those parasites, and the immunocompetence of the recipient. For viscerotropic leishmaniasis, the prevalence of latent infection in returning soldiers is unknown. Limited serosurvey results of three military units in which cases of viscerotropic *L. tropica* were found showed a 5% (23 of 471) incidence of a single postdeployment titer $\geq$ 1:32 by indirect immunofluorescence (Grogl M, Magill AJ, unpublished data). Although the eight patients noted above had a lower parasite burden than patients with kala-azar, isolation of the parasite from the bone marrow indicates that they are parasitemic. Interestingly, intracellular amastigotes were detected in the blood of a patient with viscerotropic *L. tropica* (Grogl M, Magill AJ, unpublished data). This observation is probably significant, considering that we were able to study the blood of only one of the eight patients with viscerotropic leishmaniasis. We have demonstrated that *Leishmania* can survive and maintain infectivity in blood under blood bank conditions. Unfortunately, transmission of even a small inoculum into multiply transfused, immunosuppressed, or critically ill patients may lead to a nonspecific, fulminant, febrile illness.

Kala-azar represents the most severe manifestation of the full spectrum of visceral infection

caused by *Leishmania*. However, infection with *L. donovani* and viscerotropic *L. tropica* most frequently results in asymptomatic infection, acute febrile illness, or prolonged, nonspecific, systemic illness that does not progress to kala-azar. Epidemiologic studies have shown that the ratio of infection to disease ranges from 6.5:1 in Brazil[16] to 30:1 in Kenya.[17] Humans once infected probably remain so for life. A good reason for believing that infection is lifelong is the increasing number of cases in which clinical infection can only be satisfactorily explained by reactivation of latent disease in patients who are immunosuppressed by chemotherapy or human immunodeficiency virus infection.[18–20]

The problem for the transfusion medicine community is the challenge of providing safe blood components in a changing environment. An example of blood bank procedures that are appropriate are ones to prevent transfusion-related *Plasmodium falciparum* infection: a person may donate blood only if he or she has not had symptoms for three years after exposure.[21] However, these standards have already been challenged as inappropriate for a chronic, less symptomatic disease such as *P. malariae* infection.[21] We think that present standards that are based on lack of symptoms for a relatively short period of time are inappropriate for a chronic, paucisymptomatic disease such as leishmaniasis, unless it is demonstrated that only symptomatic patients harbor parasites in blood. Meanwhile, a significant number of donors come from or visit leishmanial-endemic areas every year, but no adequate mass screening procedures are available. Perhaps such donors would have to be placed on a lifelong deferral, an exclusion policy that would significantly impact our donor pool. In this era of demands for zero-risk blood transfusion, there is an urgent need for determination of the prevalence of disease and potential for transmission. Blood banks will continue to be faced with the challenge of finding an acceptable balance between two competing objectives, the limitation of transfusion-transmitted diseases and the assurance of an adequate supply of high-quality blood. These cost-benefit considerations signify that existing medical and blood bank screening techniques are unlikely to screen out the presence of microorganisms such as *Leishmania*, which can be found in blood, can survive blood bank storage conditions and are infective. However, not until these questions are answered (prevalence of the disease in our donor population and potential for transmission) should we hold that the blood bank procedures to rule out leishmaniasis are scientifically justified.

Transfusion-associated *Trypanosome cruzi*,[22] *Babesia microti*,[23, 24] *P. falciparum*,[25] *P. vivax*,[25] *P. malariae*,[25] and *P. ovale*[25] have been demonstrated in the United States. As our study indicates, leishmaniasis should be included in the differential diagnosis of transfusion-associated parasitic infections.

The premise underlying this report, that both viscerotropic *Leishmania* (*L. donovani* complex) and dermatotropic *Leishmania* (*L. tropica*,[1] *L. braziliensis*,[13, 26] *L. amazonensis*[27]) can visceralize and contaminate blood, leads to a fundamental question: the factors resulting in the hematotropism of the parasite. Active areas of future study are the kinetics and frequency of peripheral blood parasitemia in humans (donors), the type (subpopulation) of highly phagocytic host cells and derivatives involved, and the characteristics (markers) of the parasites.

Disclaimer: The opinions or assertions herein are the private views of the authors and are not to be considered as official or as reflecting the views of the Department of Defense.

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REFERENCES

1. Magill AJ, Grogl M, Gasser RA, Wellington S, Oster CN, 1993. Viscerotropic leishmaniasis caused by Leishmania tropica in soldiers returning from Operation Desert Storm. *N Engl J Med 328:* 1383–1387.
2. Chung HL, Chow KK, Lu JP, 1948. The first two cases of transfusion kala-azar. *Chin Med J 66:* 325–326.
3. Andre R, Brumpt L, Dreyfus B, Passelecq A, Jacob S, 1958. Cutaneous leishmaniasis, cutaneous-glandular leishmaniasis and transfusional kala-azar. *Trop Dis Bull 55:* 379–381.
4. Kostmann R, Barr M, Bengtsson E, Garnham PCC, Hult G, 1963. Kala-azar transferred by ex-

change blood transfusion in two Swedish infants. *Proceedings of the Seventh International Congress of Tropical Medicine and Malaria.* Volume 2, 384.
5. Cohen C, Corazza F, De Mol P, Brasseur D. 1991. Leishmaniasis acquired in Belgium. *Lancet 338:* 128.
6. Gupta BMD. 1930. The diagnosis of kala-azar by culture of the peripheral blood. *Indian Med Gaz 65:* 489–492.
7. Knowles R, Gupta BMD. 1924. The diagnosis of kala-azar by examination of thick blood films. *Indian Med Gaz 59:* 438–440.
8. Chulay JD, Adoyo MA, Githure JI. 1985. Leishmania donovani parasitemia in Kenyan visceral leishmaniasis. *Trans R Soy Trop Med Hyg 79:* 218–222.
9. Grogl M, Oduola AMJ, Cordero LDC, Kyle DE. 1989. Leishmania spp. I: Development of Pentostam-resistant clones in vitro by discontinuous drug exposure. *Exp Parasitol* 69: 78–90.
10. Jackson PR, Pappas MG, Hansen BD. 1985. Fluorogenic substrate detection of viable intracellular and extracellular pathogenic protozoa. *Science 227:* 435–438.
11. U. S. Department of Health and Human Services. 1985. *Guide for the Care and Use of Laboratory Animals.* Bethesda: National Institutes of Health. Publication No. 86-23.
12. Anthony RL, Grogl M, Sacci JB, Ballou RW. 1987. Rapid detection of Leishmania amastigotes in fluid aspirates and biopsies of human tissues. *Am J Trop Med Hyg 37:* 271–276.
13. Martinez JE, Arias AL, Escobar MA, Saravia NG. 1992. Haemoculture of Leishmania (Viannia) braziliensis from two cases of mucosal leishmaniasis: re-examination of haematogenous dissemination. *Trans R Soc Trop Med Hyg 86:* 392–394.
14. Bowdre JH, Campbell JL, Walker DH, Tart DE. 1981. American mucocutaneous leishmaniasis. Culture of a Leishmania species from peripheral blood leucocytes. *Am J Clin Pathol 75:* 435–438.
15. Nuwayri-Salti N, Mu'Atassem D, Salman S, Izzedine N, Kibbi AG. 1992. Chronic cutaneous leishmaniasis: Leishmania parasites in blood. *Int J Dermatol 31:* 562–564.
16. Badaro R, Jones T, Lorenco R. 1986. A prospective study of visceral leishmaniasis in an endemic area of Brazil. *J Infect Dis 154:* 639–649.
17. Bryceson ADM. 1987. Leishmaniasis. Weatherall DJ, Ledingham JGG, Warrell DA, ed. *Oxford Textbook of Medicine.* Second edition. Volume 1. Oxford: Oxford University Press. 5.524–5.532.
18. Badaro R, Carvalho E, Rocha H, Queiroz A, Jones TC. 1986. Leishmania donovani: an opportunistic microbe associated with progressive disease in three immunocompromised patients. *Lancet i:* 647–649.
19. Alver J, Blazquez J, Najera R. 1989. Association of visceral leishmaniasis and human immunodeficiency virus infections. *J Infect Dis 160:* 560–561.
20. Montalban C, Martinez-Fernandez R, Calleja JL, Garcia-Diaz JD, Rubio R, Dronda F, Moreno S, Yebra M, Barros C, Cobo J, Martinez MC, Ruiz F, Costa JR. 1989. Leishmaniasis (Kala-azar) as an opportunistic infection in patients infected with human immunodeficiency virus in Spain. *Rev Infect Dis 2:* 655–660.
21 Sazama K. 1991. Prevention of transfusion-transmitted malaria: is it time to revisit our standards? *Transfusion 31:* 786–788.
22. Grant IH, Gold JWM, Wittner M, Tanowitz HB, Nathan C, Meyer K, Reich L, Wollner N, Steinherz L, Ghavimi F, O'Reilly RJ, Armstrong D. 1989. Transfusion-associated acute Chagas' disease acquired in the Unite States. *Ann Intern Med 111:* 849–851.
23. Mintz ED, Anderson JF, Cable RG, Hadler JL. 1991. Transfusion-transmitted babesiosis: a case report from a new endemic area. *Transfusion 31:* 365–368.
24. Popovsky MA. 1991. Transfusion transmitted babesiosis. *Transfusion 32:* 296–298.
25. Nahlen BL, Lobel HO, Cannon SE, Campbell CC. 1991. Reassessment of blood donor selection criteria for United States travelers to malarious areas. *Transfusion 31:* 798–804.
26. Pacheco-Cartin M, Cespedes-Fonseca R, Calderon H. 1961. Leishmaniosis cutanea. Lesiones hepaticas concomitantes estudiadas en biopsia con aguja de Vim-Silverman. *Acta Med Costarricence 4:* 2–23.
27. Barral A, Pedral-Sampaio D, Grimaldi G, Momen H, McMahon-Pratt D, Ribeiro de Jesus A, Almeida R, Badaro R, Barral-Netto M, Carvalho EM, Johnson WD. 1991. Leishmaniasis in Bahia, Brazil: evidence that Leishmania amazonensis produces a wide spectrum of clinical disease. *Am J Trop Med Hyg 44:* 536–546.

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